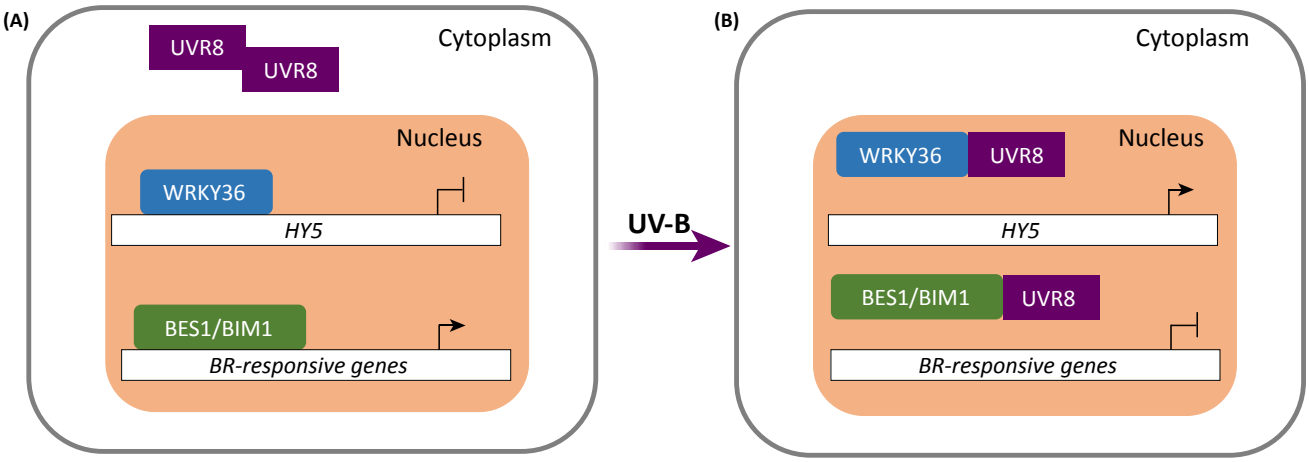

Figure 1. UVR8 interacts with different transcription factors to elicit UV-B responses. (A) Without UV-B light, UVR8 proteins gather in the cytoplasm as dimers. In the nucleus, WRKY36 and BES1/BIM1 directly repress *HY5* or induce BR-responsive gene expressions, respectively. UV-B light stimulates UVR8 monomerization and nucleus localization. (B) UVR8 monomers then interact with WRKY36/BES1/BIM1 transcription factors and abrogate their transcriptional activities, which results in the induction of *HY5* expression and suppression of BR-responsive gene expressions.

Instead of promoting HY5 degradation in darkness, COP1 enhances HY5 stability under UV-B exposure [12]. Why nucleus-localized COP1 does not target HY5 for degradation under UV-B condition is still a mystery. The UVR8–COP1 interaction is also interesting. The interaction between COP1 and either red or blue light photoreceptors is not wavelength specific, while COP1 interacts with UVR8 only under UV-B treatment [12].

Thirdly, although these new reports fill the gap between UV-B perception and transcription, they also raise new questions. (i) Both the WRKY36- and the BES1/BIM1-mediated cascade modulate cell elongation, but the relationship between these two different pathways is not clear. (ii) How does WRKY36 repress *HY5* transcription? We have not found the ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR-ASSOCIATED AMPHIPHILIC REPRESSION (EAR) repression motif in the WRKY36 protein sequence and assume that WRKY36 might interact with other co-repressors to exert its transcriptional repression activity. (iii) Based on the phenotypic observations and gene expression studies, other uncharacterized UVR8-interacting transcription factors are worthy of future exploration.

In conclusion, these recently identified UVR8-interacting transcription factors broaden our current understanding of UV-B signaling, especially through revealing the regulatory mechanisms of downstream transcription.

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Forum

Peptide Aptamers to Inhibit Protein Function in Plants

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Peptide aptamers – artificial short peptides with specific binding affinity for target molecules – can be used to interfere with protein functions and protein–protein interactions in plant cells. Therefore, peptide aptamers have emerged as a new, powerful tool with high efficiency and specificity and wide applications in functional genomics and plant biotechnology.

Proteins are key players in the control of plant life and death; thus, the exploration of protein biological functions has attracted considerable attention. It has been demonstrated that altering or impairing the activity of a target protein can inform functional genomics and has broad applications in biotechnology. This perturbation can be achieved at the DNA, RNA, and protein level. At the DNA level, emerging genome-engineering tools, particularly CRISPR–Cas9-based methods, have been widely used to knock out or alter protein functions; at the RNA level, RNAi can be used to knock down protein expression by post-transcriptional mRNA silencing. However, these techniques affect protein functions by impairing gene structure or expression.

In recent years, artificial small molecules such as aptamers have emerged as effective techniques to directly affect protein function [1,2]. The term aptamer, derived from the Latin *aptus* (fitting) and Greek *meros* (part), describes small molecules (proteins or nucleic acids) that are generated by combinatorial approaches and screened for specific binding to a target protein or other molecules. Nucleic acid aptamers are short (about 25–90 nt), single-stranded DNA or RNA sequences with typical structural motifs including stems, loops, purine-rich bulges, or hairpin structures and their biological functions depend on their length and environmental conditions [3]. Nucleic acid aptamers are increasingly used for monitoring food safety and medical diagnostic applications, because of their specific binding characteristics and minimal modifications.

Peptide aptamers are short (8–20 amino acids) peptides that display a peptide loop in an inert scaffold protein with highly specific binding affinity for a given target. Peptide aptamers can specifically bind and inactivate a target protein, thus serving as strong competitive inhibitors. They can specifically block the formation of protein–protein interactions and interfere with gene function without altering protein levels or structure. Work on peptide aptamers has focused on targeting proteins rather than the wide range of biomolecules targeted by nucleic acid aptamers. In contrast to antibodies, peptide aptamers are much lower in molecular weight and possess higher binding affinity and recognition properties for their targets *in vivo* and *in vitro*. To date, peptide aptamers have shown wide application prospects in clinical treatment and animal gene functional verification. For example, peptide A17 derived from yeast two-hybrid (Y2H) screening can specifically inhibit HSP70 activities by binding to the ATP-binding domains and induced the regression of subcutaneous tumors [4]. Yeh *et al.* reported the use of a peptide aptamer interference (PAPTi) approach for structure–function network studies. They identified peptide aptamers against Dsh and β-cat by phage display to target the Wnt signaling pathway and demonstrated that FNDY peptides can be used to perturb protein activities [5]. In recent years, peptide aptamers have also been developed and successfully applied to 'reverse genetics' to illuminate the function of a given gene product in plants [1,6].

Peptide aptamers are generally produced as libraries of random peptides that are then screened for specific binding. The peptide aptamers that specifically bind to target proteins can be identified by various selection strategies, like phage, ribosome, mRNA-display, and Y2H assays [7]. The mechanisms of perturbation of peptide aptamers are mainly through the competition with its native partners, as most proteins form homo- or heterocomplexes in cells, like the exon junction complex (EJC) proteins MAGO NASHI (MAGO) and Y14 (Figure 1A) [8]. The peptide aptamers of MAGO can be achieved via the screening of a random short-peptide library (i.e., using a Y2H strategy) (Figure 1B). The strength and specificity of the aptamer–protein interaction and the interacting domains remain unclear; therefore, it is necessary to use *in vitro* or *in vivo* analyses to confirm the biological detection using multiple approaches, such as Y2H, pull-down, and bimolecular fluorescence complementation (BiFC) assays [1]. The obtained optimal peptide aptamers that specifically bind either interacting protein can disrupt the formation of the complex and this peptide can thus be used to analyze protein function (Figure 1C). The optimal peptide aptamers once overexpressed can competitively bind to the target protein *in vivo* and *in vitro*, thus disturbing the native protein–protein interaction (Figure 1D). Besides the traditional plant genetic transformation [1], two additional strategies can be used to deliver the peptide aptamers: artificial synthesis and root-zone irrigation [6] and cell-penetrating peptide (CPP)-mediated techniques [7,9] (Figure 1E).

Peptide aptamers have been developed and successfully applied to reverse genetic approaches for efficient and precise perturbation of protein function in plants [1,6]. For example, Song *et al.* developed an antagonistic peptide to functionally dissect CLAVATA3/ENDOSPERM SURROUNDING REGION (ESR)-related (CLE) family members in *Arabidopsis thaliana*. The authors established an efficient interference system by culturing seedlings in nutrient solution containing the artificial synthetic peptide [6]. More recently, Gong *et al.* introduced a peptide aptamer targeting the EJC core component MAGO-Y14

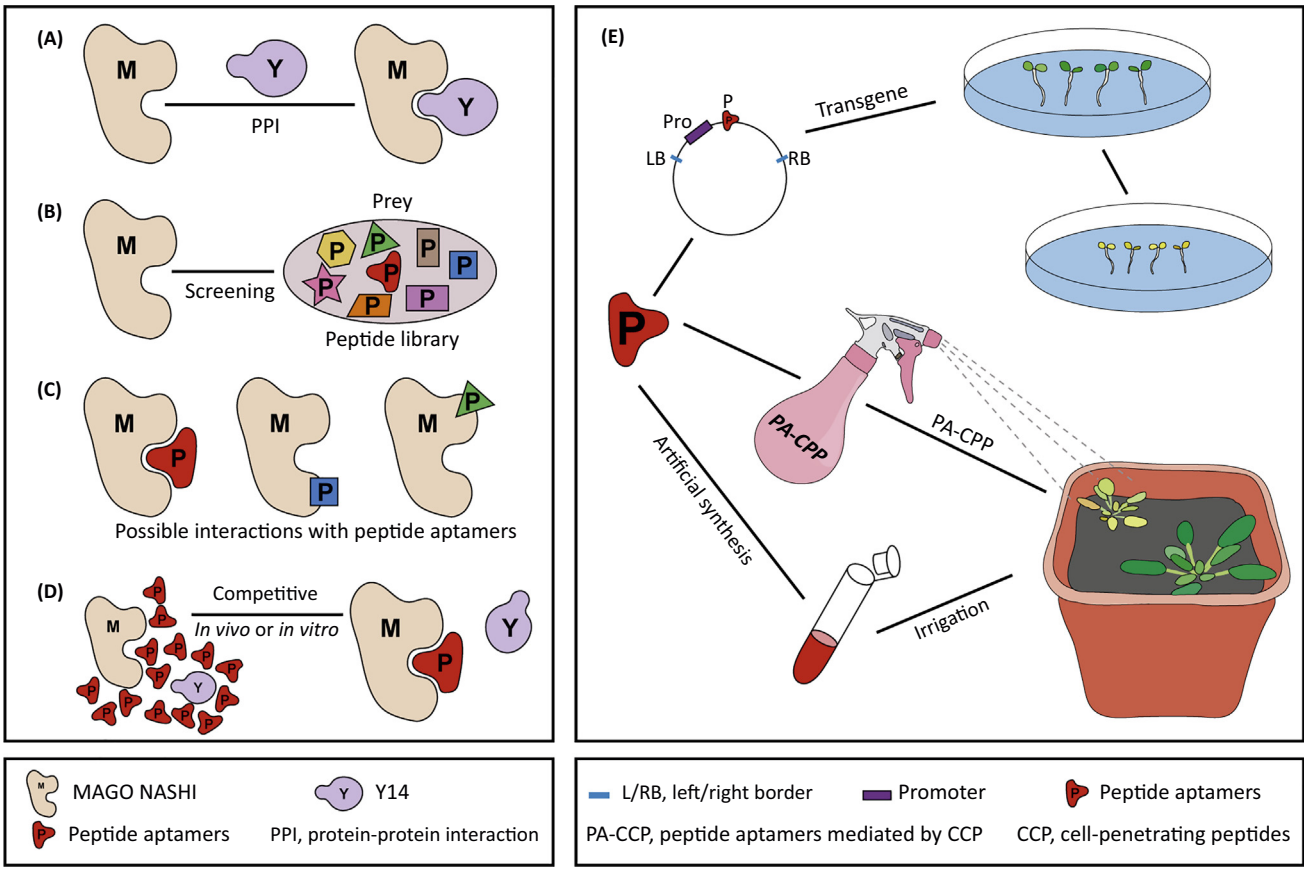

Figure 1. Generation and Applications of Peptide Aptamers in Plant Biotechnology. (A) Exon junction complex (EJC) core proteins MAGO NASHI (M) and Y14 (Y) form an obligate heterodimer in plant cells [8]. (B) Screening of a peptide aptamer library using protein M as the bait. (C) Various peptide aptamers (P) that interact with the target protein M are obtained. Some interact with the site for M–Y interaction (red) and some interact with other parts of M (blue or green). The aptamers highlighted in red are likely to be optimal, having the capability to specifically and strongly interact with M. (D) The optimal peptide aptamers (red) once overexpressed can compete with Y for specific binding to M, thus impairing M–Y heterodimerization and protein function. (E) Delivery of the peptide aptamers into plants involves conventional transgenic delivery of a construct expressing the aptamer (P) driven by a suitable promoter (Pro) that can be constitutive, inducible, or tissue specific depending on the application [1]. A peptide aptamer CPP-mediated (PA-CPP) delivery strategy [7,9] and direct artificial synthesis and irrigation of the peptide aptamers [6] are alternatives to genetic transformation procedures.

into rice (*Oryza sativa*) [1,8]. They obtained a specific aptamer named PAP that antagonizes rice MAGO by screening a 16-amino-acid random peptide library. Pull-down and BiFC analyses showed that PAP specifically and competitively interacts with the rice MAGO protein, interfering with the MAGO-Y14 interaction [1]. Moreover, PAP transgenic rice plants displayed similar or more serious defects compared with the rice *MAGO* and *MAGO-Y14* RNAi lines [1,10], indicating that competitive binding of PAP to MAGO can destroy the function of the native MAGO-Y14 heterodimer in rice [1]. Furthermore, investigation of PAP and GFP-PAP interactions showed that fusion of the peptide aptamer with the in-frame neutral scaffold protein is not necessarily required [1].

In contrast to reverse genetic approaches that target a specific protein, Bao *et al.*, recently established a new forward genetic approach to identify novel molecules that can modulate important processes in plants by transforming a library expressing peptide aptamers of six or twelve random amino acids into *Arabidopsis* and then screened the transgenic plants for phenotypes of interest. This approach provides a set of novel peptides that affect photosynthesis, flowering, red-light responses, and other processes [2].

Peptide aptamers can also be applied to produce broad-spectrum viral resistance in plants. For example, based on the evolutionary conservation of the N-terminal protein of tospoviruses, Rudolph *et al.* generated *Nicotiana benthamiana* transgenic lines overexpressing a 29-

amino-acid peptide that strongly interacts with the nucleocapsid proteins (N) of various tospoviruses; the transgenic plants display strong resistance to at least five viruses [11]. Reyes *et al.* generated broad-spectrum antigeminivirus peptide aptamers that target a highly conserved replication initiator protein (Rep/AL1) in *N. benthamiana* [12]. The authors further demonstrated that peptide aptamers can be extended to produce virus resistance in tomato (*Solanum lycopersicum*) [12].

Collectively, recent studies indicate that peptide aptamer interference provides an alternative, complementary, and direct approach for plant functional genomics. Peptide aptamers can perturb protein function directly at the protein level, serving as a supplementary strategy for other tools that act at the DNA or RNA level. We expect that in the future many more peptide aptamers will be generated and optimized. Furthermore, these advances will contribute to expanding the molecular toolbox of plant biotechnology for the improvement of crop traits.

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